

Expression of tissue factor by melanoma cells promotes efficient hematogenous metastasis

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Communicated by M. Frederick Hawthorne, September 21, 1992

ABSTRACT Metastasis is a multistep process which requires highly adapted interactions of tumor cells with host target organs. Compared with nonmetastatic cells, metastatic human melanoma cells express 1000-fold higher levels of tissue factor (TF), the major cellular initiator of the plasma coagulation protease cascades. To explore whether TF may contribute to metastatic tumor dissemination, we analyzed the effect of specific inhibition of TF function on human melanoma metastasis in severe combined immunodeficient (SCID) mice. Using species-specific antibodies to TF, we demonstrate that initial adherence is insufficient for successful tumor cell implantation in a target organ. Rapid arrest of human tumor cells in the lungs of mice was not diminished by inhibition of TF. However, inhibition of TF receptor function and consequent reduction in local protease generation abolished prolonged adherence of tumor cells, resulting in significantly reduced numbers of tumor cells retained in the vasculature of the lungs. The growth of pulmonary metastases was also significantly inhibited by a blocking anti-TF monoclonal antibody and Fab fragments thereof, whereas a noninhibitory antibody lacked antimetastatic effects. Cell surface expression of functional TF thus contributes to melanoma progression by allowing metastatic cells to provide requisite signals for prolonged adhesive interactions and/or transmigration of tumor cells across the endothelium, resulting in successful metastatic tumor implantation.

Tumor cells engage in various highly adapted interactions with the host to accomplish the multistep process of metastasis. This involves access of tumor cells to the vasculature by local invasion and entry into the vascular compartment, followed by arrest and implantation of tumor cells in the capillary bed of target organs and subsequent proliferation into metastases (1). Melanoma metastasis is a paradigm (2) to analyze the molecular pathways of hematogenous metastasis. Clinically, melanoma metastasizes from the skin to a variety of organs (3), including the lungs. This path of metastasis is recapitulated with many human melanoma cell lines when introduced intravenously into immunodeficient mice (4, 5). Molecular participants in the metastatic process include adhesion receptors expressed on tumor cells (6), as well as host factors, such as constitutively expressed organ-specific (7) or inducible (8) endothelial cell adhesion molecules.

Efficient metastasis of tumor cells has long been suggested to be possibly dependent on the plasma coagulation cascades (9, 10). Rather nonselective anticoagulants such as warfarin and heparin can inhibit experimental metastasis of certain tumors in animals (11, 12). Studies with the highly specific thrombin inhibitor hirudin suggest that generation of the pleiotropic serine protease thrombin may trigger essential events during metastasis of melanoma cells (13). However, other serine proteases of the coagulation system, such as

factor VII, have also been suggested as potentially important host factors for metastasis (14). Indeed, factor VII at the site of implantation may bind to tissue factor (TF), its receptor and catalytic cofactor. TF can be expressed on host endothelial cells in response to induction by a tumor-secreted cytokine, vascular permeability factor (15, 16). Thus, the formation of an initiating TF-factor VIIa protease complex on host cells may locally trigger coagulation as an important step for tumor cell implantation. However, earlier studies demonstrated expression of TF on a variety of tumor cell lines (17-19), indicating that host cell TF may not be required at the local site of tumor cell arrest in the vasculature.

TF is a single-chain, 263-amino acid membrane glycoprotein, and its primary sequence indicates structural similarity with the cytokine receptor family (20). The TF extracellular domain binds factor VIIa and serves as the catalytic cofactor for this serine protease, thereby initiating the plasma coagulation protease cascades (20). Although alternative pathways exist to activate coagulation, including a tumor cell protease designated cancer procoagulant (21), the *in vivo* activation of this protease system predominantly proceeds via the TF pathway (22). TF is typically not expressed to any significant degree on cells of the blood or the endothelium, but is constitutively expressed on certain cell types with a differentiation-dependent regulation (23). Although correlative evidence suggests that metastatic tumor cells often express TF, it has not been established whether the expression of TF is an epiphenomenon or is indeed required for the metastatic phenotype. Analysis of TF expression on melanoma cells is demonstrated here to follow the progression from normal melanocytes toward melanoma cells with a metastatic phenotype. Further, we show in a xenograft model that the selective inhibition of TF function on the surface of melanoma cells abolishes prolonged adherence of these cells in the microvasculature and inhibits pulmonary metastasis, suggesting a functional importance of TF expressed on tumor cells rather than on host cells during the metastatic process.

MATERIALS AND METHODS

Cell Lines. Normal human epidermal melanocytes (Clonetics, San Diego) were maintained in melanocyte growth medium (Clonetics) and used prior to the 10th passage. The human melanoma cell line WM35 (24) was provided by M. Herlyn (Wistar Institute). WM35 was maintained in MCDB153/L15 medium with insulin (5 μ g/ml) and 2% fetal bovine serum (FBS). The human melanoma cell line M24met has been described in detail (25). The M24met line was derived from M24 (26) provided to us by D. L. Morton (University of California, Los Angeles). The human melanoma cell line C8161 (27) was provided by M. J. C. Hendrix (University of Arizona). M24met and C8161 were maintained in RPMI 1640 supplemented with 10% FBS. WM35 and M24met were cultured on tissue culture plastic that was

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Abbreviations: TF, tissue factor; mAb, monoclonal antibody.

pretreated with 1% gelatin. All cells were routinely tested for mycoplasma infection.

Monoclonal Antibodies (mAbs). The murine anti-human TF mAbs TF8-5G9 and TF9-10H10 have been characterized (28, 29). Both mAbs are of IgG1 isotype. The IgG1 mAb to the simian virus 40 large T antigen (TIB115, American Type Culture Collection) was used as isotype-matched irrelevant control. Fab fragments of mAb TF8-5G9 were produced by papain digestion (28) followed by ion-exchange chromatography.

Functional Analysis of TF. TF functional activity was analyzed in a plasma coagulation assay after octyl glucopyranoside lysis of cell pellets (30). TF was quantified by converting clotting times into procoagulant activity units, based on a calibration curve for purified and phospholipid-reconstituted recombinant TF. The activity which yielded a 50-sec clotting time was defined as 1 unit/ml. The procoagulant activity was demonstrated to be TF-specific by blocking TF-function in the cell lysates by a 30-min preincubation with mAb TF8-5G9 (30 μ g/ml). The functional activity of human recombinant TF was compared in mouse plasma (Sigma) and pooled normal human plasma by using a standard coagulation assay.

Analysis of Cell Surface TF. Cell surface expression of TF was analyzed by flow cytometry. Melanoma cells (10^6 cells per tube) were suspended in phosphate-buffered saline (PBS) with mAb (50 μ g/ml) for 45 min on ice. Cells were washed, and bound primary mAb was detected by incubation for 45 min on ice with goat anti-mouse IgG antibody labeled with fluorescein. After washing, cells were analyzed with a FACS 440 flow cytometer (Becton Dickinson). Cell surface TF was quantified on M24met melanoma cells by radioligand analysis. The mAb TF8-5G9 was labeled to a specific activity of 5 nCi (185 Bq)/ng with Na¹²⁵I (Amersham) by the use of Enzymobeads (Bio-Rad). Radioligand binding analysis with the radiolabeled mAb was performed on melanoma cell monolayers (31).

Experimental Metastasis Assay. Human melanoma cells (0.5 – 1.0×10^6 cells in 200 μ l of PBS) were injected into the lateral tail vein of 6- to 8-week-old female C.B-17 *scid/scid* mice. Prior to injecting, cells were incubated for 45 min at room temperature with a total of 1 mg of mAb or Fab fragment. Cells for a control group were incubated in PBS only. When a 2-mg total dose was administered, mice received a second intravenous injection of 1 mg of mAb after 24 hr. Twenty-one days later, these animals were sacrificed and necropsy was performed. All major organs were systematically examined for the presence of tumors. The lungs were removed and their weight was determined prior to fixation in Bouin's solution. Tumor foci on the lung surface were counted under a low-magnification microscope.

Distribution of Radiolabeled Melanoma Cells After Intravenous Injection. The initial localization of radiolabeled tumor cells to organs was analyzed as described (13). Cells were cultured for 24 hr in the presence of 5-[¹²⁵I]iodo-2'-deoxyuridine (Amersham) at 0.1 μ Ci/ml, resulting in the incorporation of 50,000 cpm per 10^6 M24met cells. This had no effect on the viability of the cells. After incubation with mAb or saline for 45 min, 10^6 cells in 200 μ l were injected into the lateral tail vein of female *scid/scid* mice. Mice were bled from the orbital sinuses (50 μ l per mouse) and sacrificed at 10 min, 90 min, 240 min, and 24 hr after injection. Lungs, livers, spleens, and kidneys were collected and washed in 70% ethanol for 3 days prior to γ counting.

RESULTS

Metastatic Melanomas Express High Levels of TF. The expression of TF was analyzed by a coagulation assay. The procoagulant activity of the cell lysates was shown to be

TF-specific by inhibition with anti-TF mAbs (Fig. 1). Both cultured human melanocytes and the cell line WM35, which was derived from a primary nonmetastatic melanoma (24), expressed TF at very low levels. In contrast, the melanoma lines M24met (25) and C8161 (27), which were established from melanoma metastases, had a >1000-fold higher level of TF expression. When cell lysates of M24met and C8161 were electrophoretically separated and analyzed by Western blotting with monospecific antibodies to TF, a single 47-kDa protein was identified under reducing conditions (data not shown), consistent with fully glycosylated and properly processed human TF (32). High-level expression of TF thus characterizes these metastatic melanoma cells.

Indirect immunofluorescence was used to assess whether TF was expressed on the tumor cell surface or was sequestered in intracellular compartments where it should be of minor functional significance *in vivo*. Specific immunoreactivity of mAbs TF8-5G9 and TF9-10H10 was demonstrated by flow cytometry with nonpermeabilized M24met or C8161 cells (Fig. 2). Two additional anti-TF mAbs (TF9-6B4 and TF9-9C3) to nonoverlapping epitopes provided similar results (data not shown). The cell surface expression of TF on M24met cells was 3.5- to 4-fold lower than on C8161 cells, consistent with the relative functional TF activities of these cell lines in the coagulation assay. Cell surface expression on M24met cells was also quantified by radioligand binding analysis with radiolabeled mAb TF8-5G9. Scatchard analysis demonstrated saturable binding to $64,000 \pm 28,000$ sites per cell, with a dissociation constant of 197 ± 5 pM (mean \pm SD, $n = 3$). These data are consistent with predominant cell surface expression of TF on the cells. TF therefore represents the major initiator of the coagulation pathways on the surface of these metastatic melanoma cells.

Specific Inhibition of Human TF in Murine Blood. To explore whether the presence of functional TF on the melanoma cell surface contributes to metastatic tumor dissemination, we chose a xenograft model in which human melanoma cells are injected intravenously into mice with severe combined immunodeficiency (SCID). Species compatibility was assessed with recombinant human TF reconstituted into phospholipid vesicles. Human TF triggered coagulation with the same efficiency in both human and mouse plasma (Fig. 3), demonstrating that the xenograft model adequately reflects the interaction of tumor cell TF with the host's blood. Based on analysis in a purely human system, mAbs TF8-5G9 and TF9-10H10 have distinct functional characteristics, although

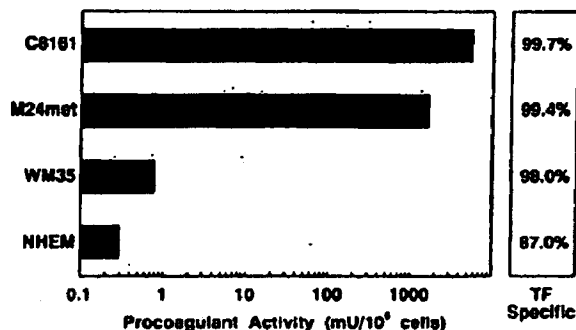


FIG. 1. TF expression by cultured human melanocytes and melanoma cell lines. TF functional activity was analyzed in a plasma coagulation assay after octyl glucopyranoside lysis of cell pellets of normal human epidermal melanocytes (NHEM) and melanoma cell lines WM35, M24met, and C8161. TF specific activity was assessed after blocking TF in the cell lysate by a 30-min preincubation with mAb TF8-5G9 (30 μ g/ml). Means from four independent experiments are shown. SDs were 223 (M24met), 1243 (C8161), 3.5 (WM35), and 0.2 (NHEM) milliunits (mU) per 10^6 cells.

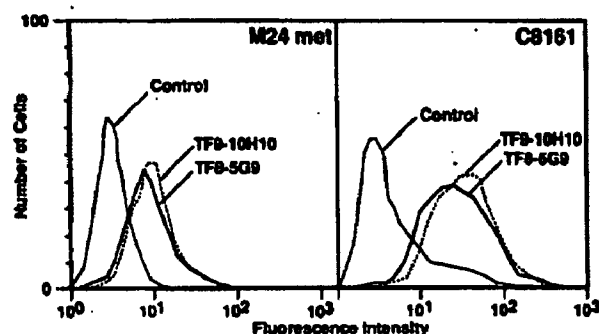


FIG. 2. Surface expression of TF on metastatic melanoma cell lines. Indirect immunofluorescence and flow cytometry were used to demonstrate TF antigen on the surface of M24met and C8161 melanoma cell lines. Cells were incubated with isotype-matched control mAb (TIB115) or anti-TF mAb TF8-5G9 and TF9-10H10, respectively. Results of a typical experiment are shown.

they are directed to partially overlapping epitopes (29). Whereas mAb TF9-10H10 does not inhibit binding of factor VIIa and function of the TF-factor VIIa complex, mAb TF8-5G9 is a potent and rapid inhibitor of human TF in human plasma due to its ability to compete with substrate access to the TF-factor VIIa complex. In addition, this mAb does not inhibit TF from various nonhuman primates (except chimpanzee) or any lower mammalian species tested to date (33), thus providing a highly specific tool to selectively block human TF function in the xenograft model. Both the inhibitory mAb TF8-5G9 and the noninhibitory mAb TF9-10H10 had identical functional characteristics whether in human or mouse plasma (Fig. 3). The use of the human TF-specific mAb TF8-5G9 thus allows selective inhibition of melanoma cell TF function without interfering with murine TF expressed on host cells.

Melanoma Cell TF Function Is Required for Hematogenous Metastasis. Intravenous injection of M24met metastatic melanoma cells into SCID mice reliably produces a large number of metastases in the lungs. The number of experimental pulmonary metastases was significantly reduced when mice were infused with M24met cells in the presence of 1 mg of TF8-5G9 in comparison with a control group injected with M24met cells in buffer (Table 1). Mice receiving TF8-5G9 had also a significant reduction in metastases when compared with animals treated with an irrelevant isotype-matched control mAb (TIB 115) or an anti-TF mAb, TF9-10H10, which binds TF with high affinity without blocking its func-

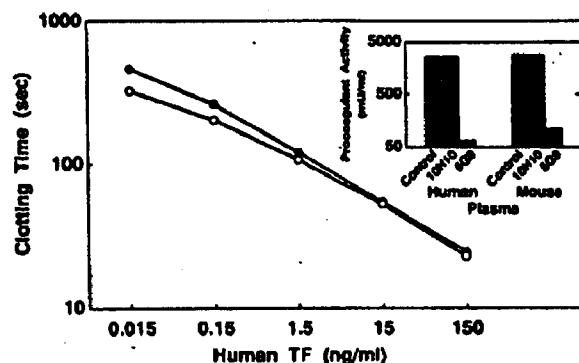


FIG. 3. Functional activity of human TF in human and mouse plasma. The ability of phospholipid-reconstituted recombinant human TF to initiate coagulation in mouse plasma (○) was evaluated in a standard clotting assay in comparison to pooled normal human plasma (●). In either case a preincubation with TF8-5G9 (30 μ g/ml), but not with TF9-10H10, inhibited TF-induced coagulation (Inset). mU, millunits.

tion. In an independent experiment the antimetastatic effect of TF8-5G9 was reproduced. Furthermore, Fab fragments of TF8-5G9 effectively inhibited M24met metastasis. This finding further excludes Fc-mediated effects, though considered unlikely because of the lack of antimetastatic effects of the anti-TF mAb TF9-10H10, which is of the same isotype as TF8-5G9. The inhibition by Fab fragments was slightly less effective than that with the intact mAb, and this may result from the 2- to 3-fold poorer affinity of Fab fragments of TF8-5G9 (28). Blocking of TF function by mAb or Fab fragments, but not binding of a noninhibitory mAb to the same receptor, reduced local protease generation and consequently reduced the number of pulmonary metastases, consistent with a requirement for TF function in hematogenous metastasis of tumor cells.

The role of TF in melanoma metastasis was further evaluated with another metastatic melanoma cell line which exhibits high levels of TF expression. Compared with buffer control, mAb TF8-5G9 significantly inhibited experimental lung metastasis of C8161 melanoma cells. Similar to M24met melanoma cells, the noninhibitory mAb TF9-10H10 did not significantly reduce melanoma metastasis. However, there was an apparent decrease in the number of pulmonary metastases when compared with the buffer control group. This statistically insignificant difference may indicate a slight effect of the 3.5- to 4-fold higher number of TF sites, and consequently

Table 1. Effect of functional inhibition of TF on experimental pulmonary metastasis

| Cells injected (no. per mouse) | mAb (total dose) | No. of metastases (total in both lungs) | P |
|---|---------------------|--|---------|
| Exp. 1, M24met (1 \times 10 ⁶) | None | 314, 353, 356, 375, 384, 402 | |
| | TF8-5G9 (1 mg) | 14, 16, 27, 42, 94, 153 | <0.0022 |
| | TF9-10H10 (1 mg) | 279, 293, 346, 363, 375, 377 | 0.31 |
| | IgG1 control (1 mg) | 281, 291, 292, 327, 368, 424 | 0.24 |
| Exp. 2, M24met (1 \times 10 ⁶) | None | 41, 86, 145, 175, 196, 221 | |
| | TF8-5G9 (1 mg) | 0, 0, 0, 0, 0, 1 | <0.0022 |
| | TF8-5G9 (2 mg) | 0, 0, 0, 0, 0, 0 | <0.0022 |
| | Fab TF8-5G9 (2 mg) | 0, 2, 5, 6, 15, 16 | <0.0022 |
| Exp. 3, C8161 (5 \times 10 ⁵) | None | 20, 21, 37, 65, 88, 170 | |
| | TF8-5G9 (2 mg) | 0, 0, 0, 2, 3, 4 | <0.0022 |
| | TF9-10H10 (2 mg) | 2, 3, 15, 21, 56, 63 | 0.09 |

M24met or C8161 cell suspensions were incubated in buffer with or without 1 mg of mAb TF8-5G9 or its Fab fragment, mAb TF9-10H10, or isotype control mAb TIB115 prior to injection. After 21 days all animals were sacrificed, their lungs were fixed, and the number of visible tumor nodules on the lungs was counted. The probability of no difference (*P*) between buffer control and mAb-treated groups was assessed with the nonparametric Wilcoxon rank sum test.

bound mAb molecules, on the surface of C8161 compared with M24met cells, which might elicit some Fc-mediated host defense mechanisms. These data demonstrate that only functional inhibition of TF results in a significant reduction of pulmonary metastasis, providing evidence that two independently established metastatic melanoma cell lines require TF function for efficient hematogenous metastasis.

TF Function Is Required for Prolonged Adherence of Melanoma Cells. The distribution of intravenously injected radiolabeled tumor cells was studied to assess whether initial arrest in various organs and persistence of tumor cells were influenced by blocking of TF function (Fig. 4). Inhibition of TF function on M24met cells had no effect on the rapid clearance of tumor cells from the blood, when compared with buffer control. As early as 10 min after injection, only 1% of infused tumor cells remained per ml of blood, and radiolabeled cells were virtually absent after 24 hr in all animals. In the livers of mice treated with the anti-TF mAb TF8-5G9, more tumor cells were initially arrested in comparison to the controls. This may reflect increased clearance of a small fraction of the antibody coated cells by the reticuloendothelial system of the liver. Only traces of radiolabel (<0.25% of the total dose) were detected in spleens and kidneys at all time points, with no differences in mAb-treated or untreated animals.

The majority of tumor cells rapidly localized to the lungs with no difference between the anti-TF mAb-treated and the untreated control group. The radiolabel in the lungs dropped from 75–80% of total tumor cells at 10 min after injection to 55–60% of the cells at 90 min. This indicates that the initial organ distribution and the selective arrest in the lung vasculature are not influenced by inhibition of TF function. However, early attachment was not sufficient to dictate successful implantation. Thus, inhibition of tumor cell TF significantly reduced the number of cells retained in the lungs 4 and 24 hr after tumor cell infusion. At 24 hr, radiolabel equivalent to 170 cells was present in lungs of animals receiving inhibitory anti-TF mAb compared with 15,000 cells in controls. Functional TF on the melanoma cells thus appeared to be required for prolonged adherence of tumor cells at the site of initial arrest.

DISCUSSION

The malignant phenotype of cells typically incorporates aberrations in growth control and their capacity to invade and metastasize. Critical events in the metastatic process are the

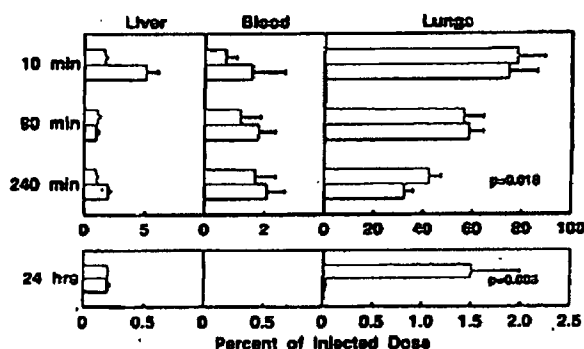


FIG. 4. Distribution of radiolabeled M24met cells after intravenous injection into SCID mice. Cells were labeled with 3-¹²⁵I]iodo-2'-deoxyuridine for 24 hr, washed, and incubated for 45 min with 1 mg of mAb TF8-5G9 (open bars) or buffer control (filled bars). Radiolabeled M24met cells were injected into the tail vein of SCID mice. Mice were bled and sacrificed at different time points. Means and SDs are based on four animals per group at each time. The probability (*p*) of no difference occurring between groups at a given time was assessed with the Wilcoxon rank sum test.

initial arrest and stable implantation of tumor cells in the microvasculature of target organs. Studies on the arrest of circulating tumor cells discriminate between an initial phase of direct contact between tumor cells and the vascular endothelium and a second phase characterized by fibrin generation and formation of a platelet thrombus in association with the arrested tumor cells (34). This indicates that proteolytic activity is rapidly generated at the site of tumor cell arrest, marking a second phase of stabilized implantation at the endothelium. We demonstrate here that generation of proteolytic activity on the surface of metastasizing human melanoma cell lines is triggered by TF, the major initiating molecule of the coagulation cascades. Indeed, the cellular expression of this molecule was observed to parallel the progression of melanoma cells to a metastatic phenotype. Whereas normal cultured human melanocytes or a primary melanoma-derived cell line had only trace levels of functional TF, an at least 1000-fold higher cell surface expression was detected on metastasizing human melanoma cells. Since all of the cell lines were proliferating, expression of TF did not reflect cell cycle differences but appeared, rather, to be the correlate of the metastatic potential.

The functional role of TF expressed on melanoma cells was explored in an experimental metastasis model in SCID mice. The mAb TF8-5G9, which selectively binds and functionally neutralizes human TF in the xenograft model, markedly reduced the number of pulmonary metastases of two metastatic melanoma cell lines. This resulted from functional inhibition of tumor cell TF rather than nonspecific host defense effects, based on (i) lack of reactivity of the murine anti-human TF mAbs with host TF, (ii) inhibition of metastasis by Fab fragments of mAb TF8-5G9, and (iii) unaltered implantation of pulmonary metastasis when a noninhibitory mAb was bound to the same cell surface receptor. Inhibition of TF function did not interfere with the initial arrest of the tumor cells in the lungs of animals but, rather, abolished the prolonged localization to this organ. This study thus establishes a requisite second phase in tumor cell implantation which is dependent on tumor cell TF-driven generation of coagulation serine proteases.

The initial arrest of tumor cells appeared to be not dependent on the generation of proteolytic activity. Direct interaction between tumor cells and endothelial cells through constitutively expressed and lung-specific endothelial cell adhesion molecules, such as Lu-ECAM-1 (7), may be responsible for this early attachment. This initial arrest, though necessary for the metastatic process (35), is not sufficient to establish metastases. In a second phase, TF on the tumor cell surface is required to activate the coagulation cascades which, among other serine proteases, ultimately generate thrombin. Inhibition of this protease in the early phase of tumor seeding has indeed been shown to reduce pulmonary metastasis (13). However, the metastatic process may be dependent on various biological effects of thrombin that are mediated through proteolytic conversion of plasma proteins, such as fibrinogen, or signaling to cells through proteolytic activation of the thrombin receptor (36).

Thrombin may trigger critical events in the early phase of tumor cell implantation. Stimulation of endothelial cells by thrombin results in rapid expression of P selectin (37), whose ligands are found on a variety of tumor cells (38). More delayed expression of E selectin upon stimulation of endothelial cells may contribute to additional cooperative adhesive interactions through carbohydrate ligands associated with the melanoma cells (39). The avidity of adhesive receptors is modulated by signaling molecules such as platelet-activating factor, which is synthesized by endothelial cells upon thrombin stimulation (40). Melanoma-endothelial cell interaction in metastasis may recapitulate the principles of leukocyte-endothelial cell interactions in inflammation (41).

This process is characterized by an initial, transient, and reversible adhesion mediated by selectins and is followed by more stable adherence resulting from the upregulation and functional participation of leukocyte integrins and their counter-receptors on the stimulated endothelial cell.

Additional events may be triggered by the initial generation of proteolytic activity at the site of tumor cell arrest. Fibrin generation may protect arrested cells from host defense mechanisms (42). Fibrin, which is deposited locally, serves as a matrix for the interaction of tumor cells through their adhesive receptors which may modify or induce the expression of genes relevant for invasion and tumor growth (43). Thrombin may support invasion of tumor cells by degrading the subendothelial matrix either directly (44) or indirectly by inactivation of inhibitors for other tumor cell-associated proteases (45). Proteolytic activation of the thrombin receptor (36) on tumor cells or host cells may modify their cellular responses and stimulate proliferation. Platelets are deposited upon thrombin stimulation at the sites of tumor cell arrest, and the inhibition of platelet function results in inhibition of metastasis in several experimental models (46, 47). However, it is not known whether the stabilization of the initial fibrin clot (48) or the release of pleiotropic growth modulators such as platelet-derived growth factor, platelet factor 4, or transforming growth factor β (49) may contribute to prolonged tumor cell survival and growth. Thus, locally generated proteases may influence additional events following the secondary stabilized localization of tumor cells.

It is not known whether occupancy of TF by ligand (factor VII or VIIa) provides additional signals to the tumor cell that favors survival and proliferation. Binding of mAb TP8-5G9 to TF not only rapidly abolishes function but also decreases the binding of ligand (28). The cytoplasmic domain of TF has been found to be phosphorylated by a protein kinase C-dependent mechanism, indicating that TF is associated with other molecules involved in signal transduction (50). In analogy to other members of the cytokine receptor family, ligand-induced dimerization of TF may provide adaptive or proliferative signals that support, or may even be required for, the metastatic process. Our findings establish a requirement for ligand binding and function of TF expressed on the tumor cell surface. Specific functional inhibition of this receptor molecule markedly reduces pulmonary metastases, providing evidence that locally generated proteolytic activity at the site of tumor cell arrest is a key event in metastatic implantation.

We thank Dorothy Martowitz and Pablo Tejada for technical assistance. This work was supported by grants from the National Cancer Institute (CA42508) and the National Heart, Lung, and Blood Institute (FO1 HL16411) and by a fellowship from the American Heart Association, California Chapter (W.R.). This is publication 7357-IMM from the Department of Immunology, The Scripps Research Institute.

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